CHEMICAL COMPOSITION OF THE OTOLITHS OF THE SEA BASS (*DICENTRARCHUS LABRAX* LINNAEUS, 1758) (PISCES, SERRANIDAE)

by

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ABSTRACT.– The chemical composition of the otoliths of the sea bass has been determined, and special reference made to possible chemical variations during their life span.

Differences between the organic composition of the otoliths of juvenile and adult fish were found, and included the protein contents and the aminoacid composition. Aragonite, the crystaline component was found in all the age groups studied. The aminoacid composition of the sea bass otoliths seems to indicate that the organic matrix could have different roles depending on the time of its formation.

RÉSUMÉ. – La composition chimique des otolithes du bar a été étudiée par l'auteur qui a souligné les variations chimiques possibles au cours du cycle biologique de l'espèce.

Des différences ont été mises en évidence entre la composition organique des otolithes des juvéniles et celle des adultes, en particulier en ce qui concerne la teneur en protéines et en amino-acides. L'aragonite, le composant cristallin, est présent dans tous les groupes d'âges étudiés. La composition en amino-acides des otolithes du bar semble indiquer que la matrice organique pourrait jouer un rôle variable selon l'époque de sa formation.

Key-words : otoliths, composition, Pisces, Serranidae, *Dicentrarchus labrax*.

Teleost fish otoliths have been shown to be composed of calcium carbonate, crystallised in the form of aragonite (Carlstrom, 1963) and a low concentration of organic material composed of protein, rich in aspartic and glutamic acids and small amounts of aromatic and basic aminoacids, described as otolin by Degens *et al.* (1969).

Seasonal variation in the organic component gives rise to the formation of concentric rings. These rings are composed of 0,22 to 0,26 % in weight of organic matter for the refringent opaque rings and 0,15 to 0,18 % organic matter for the translucent rings (Mugiya, 1964). Their annual formation enables the age of a fish to be determined from its otoliths (Blacker, 1974).

The otoliths of sea bass (*Dicentrarchus labrax*, Linnaeus, 1758) are composed of a wide nucleus, formed during the period between hatching and their first winter, and, alternately, opaque and translucent rings, seasonally laid down around the nucleus. The density and spacing of these growth rings decrease with age; the nucleus remaining the most dense part of the otolith (Morales, 1984).

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The present paper deals with the chemical composition of the sea bass otoliths, with special reference to their possible variation during the fishes life span. Such information is important when otoliths are used for age determination studies and for the understanding of the structural properties during ontogeny of their mineralized tissues.

MATERIAL AND METHODS

The otoliths studied were obtained from sea bass off the Catalonian coast (Spain) and from specimens of known age reared in the fish farm at Torre la Sal (Castellón, Spain).

The otoliths were extracted, cleaned with distilled water and stored dry until they were analyzed.

The cristalline composition of otoliths from specimens of 6 months, 1 and 4 years old was determined by X-ray diffraction. Subsamples of 0.1 g weight of dehydrated and pulverized otoliths were subdued to the action of the X-ray beam in a Philips PW 1001 diffractometer. The diffraction pattern was recorded by means of Cu radiation in one Gunier de Wolf camera and passed through a Nonius quartz monochromator.

The proportion of the organic component was determined in 23 otoliths from specimens of 8 to 61 cm in length. The concentration of the total protein nitrogen content was determined by the Microkjeldahl method (Lepper, 1950). The total protein concentration was calculated by multiplying the nitrogen content by 6.25 (the average nitrogen content in proteins).

The aminoacid composition of the otoliths was determined in 5 samples composed of 5 otoliths each from specimens 4 months (sample A1), 6 months (samples A2, A3, A4) and 4 years old (sample A5).

The samples were then hydrolized and the aminoacide composition determined with a Beckman aminoacid autoanalyzer.

RESULTS

The crystallographic studies confirmed that the sea bass otoliths are composed of aragonite (Fig. 1) with no differences found in the crystallographic composition of otoliths from fish of different age groups. It can thus be concluded that aragonite is the crystalline constituent of the otoliths throughout their life span.

The proportion of protein in the otoliths showed a large range of variation within each age group considered (Table I) and was consequently related to fish length.

The protein content in the less than 1 year old group was 10 times higher than in the other age groups. The degree of variation within age groups is relatively high. Nevertheless, the two variables are significantly correlated. The equation re-

lating the two variables is as follows :

$$y = 1.73\, x^{-0.74} \qquad y = \%\ \text{protein},\ x = \text{age in months}$$

The aminoacid composition of the otoliths is summarized in Table II. Acidic aminoacids are abundant, followed by non-polar and hydrophobic aminoacids corresponding to the composition of the otolin as described by Degens *et al.* (1969).

Some differences were found between samples, mostly in the presence of tyrosine and cysteine. In order to determine if the discrepancies in the composition of the 4 samples from young specimens were significant, a variance analysis test was applied (Table II). The results showed that the variations are randomly produced ($F = 15.38 < F_{.05,45}$).

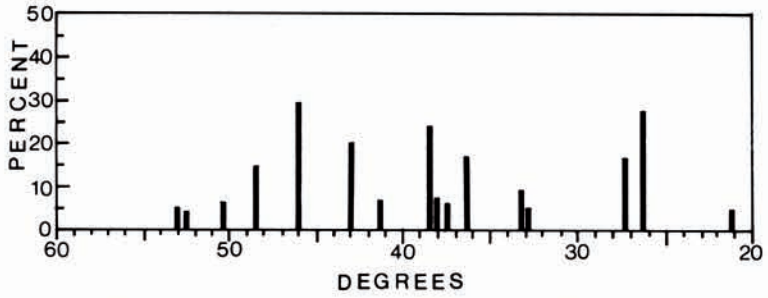

Fig. 1. – X-ray diffraction pattern for sea bass otoliths corresponding to a monomineralic aragonite composition.

Table I. – Protein content in the otoliths from various age groups.

Age	Length cm	% protein	n°	σ n-1
< 1 yr	8.0	1.26	3	0.23
1	13.7	0.11	4	0.05
2	25.5	0.13	2	0.04
3	30.2	0.11	6	0.02
4	38.0	0.11	6	0.05
5	43.0	0.16	1	0
6	53.0	0.16	1	0

DISCUSSION

The crystalline component of the sea bass otoliths is aragonite with no changes related to their age.

The protein in the adult sea bass otoliths is present in similar concentrations and with the same aminoacid composition as otolin (Degens *et al.*, 1969). There-

Table II. – Aminoacid composition of otoliths from *Dicentrarchus labrax*

Sample	CIS	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	1/2 VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	ARG
A1*	0	0.09	0.04	0.09	0.10	0.03	0.10	0.09	0	0.13	0	0.07	0.14	0.02	0.03	0.03	0.02	0.02
A2*	0	0.13	0.06	0.10	0.15	0.05	0.12	0.08	0	0.05	0.04	0.03	0.07	0	0.02	0.02	0.04	0.04
A3*	0	0.13	0.06	0.11	0,14	0.05	0.13	0.08	0	0.05	0	0.03	0.06	0.03	0.03	0.03	0.04	0.03
A4*	0	0.13	0.08	0.13	0.14	0.05	0.12	0.09	0	0.04	0	0.03	0.06	0	0.03	0.04	0.04	0.04
A5+	1.30	6.00	2.80	4.10	6.30	3.10	3.20	3.40	0	1.70	0	1.20	2.20	0	0.70	0.80	1.30	1.00

* um/um total
\+ um/g

ANOVA test between aminoacid composition of samples A1 to A4

	SS	df	F
rows	1.87	3	0.01
columns	0.10	15	15.38
error	0.02	27	
total	0.12	45	

fore, it can be concluded that the protein in the sea bass otoliths is otolin.

Differences in the organic composition of the otoliths of juvenile and adult fish were found to affect the protein content and the aminoacid composition : the percentage of protein in the juvenile otoliths is 10 times higher than in the other age groups. The content of non-polar aminoacids in the juveniles is double that in adult fish, while the acidic aminoacid, as well as asparagine, glycine and serine is lower. These latter aminoacids form the soluble matrix of the molluscan shell acting as a mould in the crystallization process (Krampitz *et al.*, 1983). Another interesting difference was the presence of tyrosine in juvenile otoliths, which is related to quinone-tanned, cross-linked proteic molecules (Meenaskhi, 1971). On the other hand, sulphur containing aminoacids usually associated with the start of crystallization (Krampitz *et al.*, 1983) were rare.

It has been accepted that the organic matrix plays an important role in the processes leading to the formation of calcified tissues. It has been suggested that the growth of crystals is due to an epitaxial growth on the protein matrix (Degens *et al.*, 1969). Nevertheless, it has been presumed in recent works, that this matrix has an important role in such functions as crystal nucleation, crystal growth control and orientation; epitaxy requires more other in the matrix than has been found (Krampitz *et al.*, 1983).

The aminoacid composition of the sea bass otoliths seems to indicate that the organic matrix could have different roles depending on the time of formation. Thus, in the juvenile period, when a dense and regularly spaced matrix is formed (Morales, 1984), the crystals could grow through an epitaxy process. At a later stage, when the properties of the matrix change and a lax reticulum is formed (Morales 1984), a nucleation process could be involved in further crystal formation.

The above conclusions are similar to those reached with regard to changes in the aminoacid composition and concentration of the organic matrix found in *Merluccius capensis* otoliths (Morales, 1985).

These results may provide an insight into the calcification processes in other calcified tissues as well as otoliths.

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